SUMMARY OF IN-VITRO AND IN-VIVO DATA

GUARDIVA®
ANTIMICROBIAL HEMOSTATIC IV DRESSING
The Bard® GuardIVa® Antimicrobial Hemostatic IV Dressing is a hydrophilic polyurethane absorbent foam impregnated with chlorhexidine gluconate (CHG) and microdispersed oxidized cellulose (m•doc™). The GuardIVa® dressing was tested via *in vitro* and *in vivo* environments to characterize the antimicrobial and hemostatic properties of the dressing. Additional testing was conducted to characterize the GuardIVa® dressing’s ability to allow normal wound healing. The clinical utility of these results is unknown.

**Antimicrobial Efficacy**

The antimicrobial properties of the dressing were tested via *in vitro* log reduction and zone of inhibition assays as well as an *in vivo* skin microflora study on human subjects. In the microflora study, swabs were taken and cultured from the skin of subjects before and after standard skin prep, and after 7 day and 10 day exposures to GuardIVa® and control dressings.

The results of the *in vitro* log reduction and zone of inhibition assays found that the dressing achieved a 4 log (99.99%) reduction and sustained zones of inhibition against all microorganisms tested over a 7 day period. The microflora study found that after both 7 and 10 day periods the GuardIVa® dressing maintained the skin flora at levels equivalent to that observed immediately following preoperative skin preparation (70% isopropyl alcohol solution), whereas the control dressings demonstrated skin flora re-growth.

**Hemostatic Efficacy**

The hemostatic properties of the GuardIVa® dressing were tested via an *in vivo* animal model where the ears of rabbits were punctured and then treated with the GuardIVa® dressing or a control gauze dressing. The time to hemostasis as well as amount of blood lost were measured in the study. It was found that injuries treated with GuardIVa® had up to seven times less blood loss and stopped bleeding in less than half the time of those treated with gauze dressings.

**CHG Levels and Wound Healing**

Due to the known sensitivity reactions to CHG reported in the literature, it was desired to restrict the amount of CHG present to the minimum amount that would effectively deliver at least a 4 log reduction against clinically relevant test organisms. In *in vitro* log reduction testing was repeated at increasing CHG concentrations following 24 hour exposure to MRSA. It was found that 24 mg of CHG achieved a greater than 4 log reduction in microbial count following 24 hr exposure. It was also found that increasing levels of CHG did not correlate with increasing *in vitro* antimicrobial efficacy.

To further understand the effect of the GuardIVa® dressing on wound healing, a dermal wound healing experiment was conducted on Sprague Dawley rats. Each rat received three full dermal thickness wounds created with an 8 mm dermal punch and each wound was covered with a test sample (GuardIVa®), a control dressing (BioPatch™), or left untreated. Each wound was measured and assessed daily. The results of the study found that when compared with the Biopatch™ dressing, wounds treated with the GuardIVa® dressing healed at a rate comparable to un-treated wounds. The Biopatch™ dressing demonstrated a delay in healing when compared to untreated wounds and wounds treated with the GuardIVa® dressing.

**Product Summary**

The Bard® GuardIVa® Antimicrobial Hemostatic IV Dressing is a hydrophilic polyurethane absorbent foam impregnated with chlorhexidine gluconate (CHG) and microdispersed oxidized cellulose (m•doc™). The chlorhexidine gluconate (CHG) in GuardIVa® Antimicrobial Hemostatic IV Dressing is a well-known antiseptic agent with broad spectrum antimicrobial and antifungal activity, and m•doc™ is a proprietary hemostatic agent, which controls surface bleeding from percutaneous catheters and vascular access sites.

The dressing’s foam material can absorb up to eleven times its own weight in fluid and has a vapor permeable, non-stick backing. The GuardIVa® Antimicrobial Hemostatic IV Dressing is an adjunct to infection control measures by providing sustained IV site protection for up to seven days. The clinical utility of these results is unknown.

**Indication**

The Bard® GuardIVa® Antimicrobial Hemostatic IV Dressing is intended for use as a hydrophilic wound dressing to absorb exudate, cover and protect catheter sites. Common applications include IV catheters, other intravenous catheters and percutaneous devices. It is also indicated for control of surface bleeding from percutaneous catheters and vascular access sites.

**Mechanism of Action**

The GuardIVa® dressing controls surface bleeding through a proprietary oxidized cellulose compound. While oxidized cellulose has been used in surgical settings as a hemostat for over 50 years, the mechanism of action is not well understood. It is hypothesized that the oxidized cellulose physically interacts with blood constituents, such as platelets, and provides a structural scaffold that facilitates clot formation and helps to control bleeding at the access site.

The CHG in the GuardIVa® dressing has been shown in *in-vitro* laboratory tests to act as an antimicrobial agent against a wide range of gram positive and gram negative microorganisms, including MRSA, MRSE, VRE, and A. baumannii. Chlorhexidine is considered to be a membrane active agent that acts by disturbing the integrity of the bacterial cell membrane and results in modification of membrane permeability. Once the cell wall is damaged, chlorhexidine then crosses into the cell itself and attacks the cytoplasmic membrane.
Antimicrobial Efficacy

Log Reduction after 24 hours and after 7 days

Introduction

The in vitro modified version of AATCC Test Method 100-2004 is a quantitative, direct contact method for the evaluation of the degree of antimicrobial activity of a test article.

Methods

GuardIVa® dressing samples were tested to determine the degree of antimicrobial activity using a modified version of AATCC Test Method 100-2004, where GuardIVa® dressings were exposed to nine representative microorganisms commonly associated with catheter-related blood stream infections. For all samples a minimum initial inoculum count of 1 x 10^6 (6-log) microbes was used. After incubation, the microorganisms were eluted from the dressing by shaking in a neutralizing solution. The number of microorganisms present in solution was determined, and the percentage reduction in the population of each was calculated. The GuardIVa® dressing was tested following 24 hour exposure to test organisms, and after 7 days of simulated conditioning followed by 24 hour exposure to test organisms. In order to be considered antimicrobial, a minimum of 4 log reduction in microbial count must be observed against bacteria and fungi.

Results

The results of the log reduction testing after 24 hours and after 7 days are found in Table 1. All nine test microorganisms were reduced by at least 4 logs after both time periods.

Average Log Reduction at 24 hrs and 7 days (Table 1)

<table>
<thead>
<tr>
<th>Gram strain</th>
<th>Avg. 24 hr log reduction</th>
<th>Avg. 7 day log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (MRSA)</td>
<td>+</td>
<td>5.5</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (MRSE)</td>
<td>+</td>
<td>5.5</td>
</tr>
<tr>
<td>Enterococcus faecium (VRE)</td>
<td>+</td>
<td>5.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>5.6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>N/A</td>
<td>4.7</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>5.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
<td>4.8</td>
</tr>
<tr>
<td>Acinetobacter baumanii</td>
<td>-</td>
<td>5.6</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>N/A</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Conclusions

The GuardIVa® dressing was found to be effective at achieving at least a 99.99% (4 log) reduction against the bacteria and the yeast listed in Table 1. The antimicrobial dressing was shown to be effective at 24 hrs and sustained for 7 days.

Zone of Inhibition Testing

Introduction

Zone of Inhibition (ZOI) tests are designed to test the ability of antimicrobial agents within a dressing to inhibit the growth of microorganisms over a period of contact time. This test evaluates the test article’s ability to prevent organism growth on contacting surfaces.

Method

The Kirby-Bauer protocol was used to demonstrate the sustained antimicrobial efficacy via zone of inhibition testing over a seven day period. Overnight cultures of representative microorganisms were prepared to a minimum inoculum count of 1 x 10^7 CFU/mL and spread on freshly prepared agar plates. An individual test article was placed onto the agar plate and incubated for 24 hr at 35 – 37°C. After incubation the area of clearing under and around the test article where bacteria were not capable of growing (called the zone of inhibition) was measured and recorded. The test article was then placed on a freshly inoculated agar plate and the procedure repeated for 6 additional days.

Results

The seven day average zone of inhibition measurements can be found in Figure 1 below.

7 Day Average GuardIVa® Zone of Inhibition (Figure 1)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Average Zone of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>30mm</td>
</tr>
<tr>
<td>MRSE</td>
<td>31.5mm</td>
</tr>
<tr>
<td>VRE</td>
<td>29mm</td>
</tr>
<tr>
<td>E. coli</td>
<td>29mm</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>26.5mm</td>
</tr>
<tr>
<td>A. baumanii</td>
<td>25mm</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>28mm</td>
</tr>
<tr>
<td>C. albicans</td>
<td>25mm</td>
</tr>
</tbody>
</table>

Conclusions

The GuardIVa® dressing was found to be effective at inhibiting bacterial growth on contacting surfaces for all microorganisms tested. The antimicrobial dressing was shown to be effective at 24 hrs and sustained for 7 days.

GuardIVa® dressing is an adjunct to infection control measures by providing sustained IV site protection. The GuardIVa® dressing has not been clinically tested for its ability to reduce catheter related blood stream infections (CRBSIs).
Bacteriocidal vs. Bacteriostatic Efficacy

Introduction
Testing was conducted to determine whether the test articles were bacteriostatic (inhibited microbial growth) or bactericidal (killed microbes) with respect to relevant microorganisms. Both the GuardIVa® dressing and the Biopatch® dressing were tested over a 7 day period.

Method
Testing was conducted using the Kirby-Bauer protocol. Overnight cultures of representative microorganisms were prepared to a minimum inoculum count of 1 x 10^7 CFU/mL and spread on freshly prepared agar plates. An individual test article was placed onto the agar plate and incubated for 24 hr at 35 – 37°C. After each incubation period the area under the test article was swabbed and the swab was transferred onto a sterile agar plate and incubated.

After incubation, if growth was observed from the swabs taken from under the test article at any time over the 7 days, it indicated bacteriostatic action (slowing of microbial growth) of the antimicrobial agent. If no growth was observed over 7 days, it indicated bactericidal action (killing of microbes). Both the GuardIVa® dressing and BioPatch® dressing were tested in this study and were compared to publicly available results of silver-based antimicrobial dressings using a similar test method.

Results
The results of the testing are found in Table 2. Table 3 shows the published results of Biopatch® dressing and other silver-based antimicrobial dressings in a similar test method.

Conclusions
Both the CHG-impregnated GuardIVa® dressing and Biopatch® dressing were found to be bactericidal (no growth) against five of the microorganisms and bacteriostatic (slowed growth) against three of the microorganisms. Both GuardIVa® and Biopatch® dressings compare favorably with silver-based antimicrobial dressings.

Suppression of Skin Microflora Re-growth Following Skin Antisepsis

Introduction
The primary objective of this study was to demonstrate that the GuardIVa® Antimicrobial Hemostatic IV Dressing actively suppresses the re-growth of skin microflora following skin preparation on healthy human volunteers.

Method
This study was performed on healthy human volunteers based on the method of Maki et al. (2008). A group of 12 study subjects was selected and enrolled for testing through informed consent. All were Caucasian with an average age of 52.5 years and an age range between 25 years and 69 years. This study was conducted to assess the capacity of the test dressings (GuardIVa®) to suppress skin flora re-growth following skin antisepsis.

On study day 0, a baseline skin flora count was established from a subclavian site on each subject (Figure 2). Another subclavian test site on the same subject was treated with 70% isopropyl alcohol (IPA) for 1 minute and allowed to air dry. After air drying, a skin flora count was measured.

Randomized sites in subclavian area of each volunteer. (Figure 2)

Four other sites were treated with 70% isopropyl alcohol for 1 minute and allowed to dry as above. After antisepsis and drying, two GuardIVa® test dressings and two control dressings (polyurethane sponge with no CHG or oxidized cellulose) were applied to the prepped sites of the subjects. Test and control dressings were applied using sterile tweezers and attached by latex-free, hypoallergenic and transparent polyurethane securement dressings. All test sites were randomly selected for each subject.

After 7 days, one pair of test and control dressings was removed from the subject and skin flora counts from under each dressing were measured. After 10 days, the second pair of test and control dressings was removed from each test subject and skin flora counts from under each dressing were measured. Skin flora was measured using standard scrubbing techniques and the skin flora beneath the dressing quantified through use of a recovery solution which was then cultured on agar plates. The skin flora counts beneath the test and control dressings were compared, and were also measured against initial pre- and post-IPA antisepsis skin flora counts. The Wilcoxon paired test was used for statistical testing of the level of significance (P-values <0.05 were considered significant).
Hemostatic Properties

Introduction
Bleeding associated with catheter insertion is a common occurrence and the GuardIVa® dressing is currently the only antimicrobial IV Site dressing indicated for control of surface bleeding from percutaneous catheters and vascular access sites.

Method
The in vivo hemostatic efficacy of the GuardIVa® dressing was determined using a rabbit ear model. The study was divided into two test periods. Within test period 1 the test item (GuardIVa® dressing) was tested on the left ear of the rabbit, the right ear was used as control (Pur-Zellin® cellulose swab, HARTMANN-RICO a.s.). Within test period 2, the GuardIVa® dressing was tested on the right ear of the rabbit, and the left ear was used as a control. Bleeding was caused by puncture of a lateral ear vein with an injection needle (external diameter = 0.9 mm). On test period 1, the puncture was performed at an acral part of the ear, on test period 2 the puncture was performed at a cranial part of the ear. Distance between both punctures was 2 – 3 cm. The test and control dressings were applied immediately after the puncture wounds were made. The time from start to the end of bleeding was measured. Test items and controls were weighed before their use and immediately after cessation of bleeding.

Conclusions
• Wounds treated with the GuardIVa® dressing had up to seven times less blood loss compared to those treated with gauze dressings, demonstrating GuardIVa® dressing’s hemostatic efficacy.
• Wounds treated with the GuardIVa® dressing on average stopped bleeding in less than half the time of those treated with the gauze dressings.

Results
The results of the skin micro-flora regrowth test can be found in Figure 3.

Suppression of skin flora re-growth in healthy volunteers (Figure 3)

Conclusions
• Raw skin flora counts were dramatically reduced following disinfection of the skin with 70% isopropyl alcohol.
• The GuardIVa® dressing showed significantly lower regrowth of skin flora compared to the control at both Day 7 and Day 10 (P<0.001).
• The GuardIVa® dressing maintained the skin flora count at less than the post-prep count for the complete duration of the study out to 10 days.
CHG Levels and Wound Healing

Antimicrobial Efficacy as a Function of Increasing Levels of Chlorhexidine Gluconate (CHG)

Introduction
Due to the known sensitivity reactions to CHG reported in the literature, it was desired to restrict the amount of CHG present to the minimum amount that would effectively deliver at least a 4 log (99.99%) reduction against clinically relevant test organisms. The same log reduction test method used to test antimicrobial efficacy was performed at increasing CHG concentrations following 24 hour exposure to MRSA.

Method
The aim of these studies was to determine the antimicrobial efficacy against Methicillin-resistant Staphylococcus aureus (MRSA) of samples of polyurethane sponge containing a fixed concentration of the proprietary oxidized cellulose hemostatic agent (m-doc™), but increasing amounts of the antimicrobial agent chlorhexidine gluconate (CHG). Samples containing varying concentrations of CHG were prepared. The antimicrobial efficacy of these sponge samples was assessed against MRSA using the same AATCC Test Method 100-2004 outlined previously.

Results
Testing was conducted in triplicate, and the average minimum log reduction values are reported in Figure 6 below.

Avg. Minimum Log Reduction with Increasing Levels of CHG (Figure 6)

Conclusions
• 24mg of CHG was found to achieve at least a 4.7 log reduction in microbial count following 24 hour exposure.
• There was only a 0.3 log difference between the minimum log reduction at 24mg and 65mg of CHG.

Wound Healing Study

Introduction
To understand the effects of the GuardIVa® dressing and Biopatch® dressing on wound healing, an in vivo dermal wound healing study was conducted in a rat model. Both the GuardIVa® dressing and Biopatch® dressing were studied with untreated sites serving as control.

Method
A dermal wound healing experiment was conducted in 12 Sprague Dawley rats. Each rat received three full dermal thickness wounds created with an 8 mm dermal punch. Following wound creation, each of the three wounds on each animal was covered with a GuardIVa® dressing, a Biopatch® dressing, or left untreated. The wound sites on each animal were covered with a secondary dressing. The wounds were uncovered (treatment dressing removed) daily for wound measurements and photographs. The same dressing that was removed was replaced on the wound after each measurement had been taken. Dressings were changed as necessary depending on the degree of saturation with exudate and wear time was limited to a maximum of 7 days exposure of a single treatment on the wound.

Results
After 7 days, the average untreated wound was found to be 76% healed. The average wound treated with a GuardIVa® dressing was found to be 66% healed. There was no statistically significant difference (p> 0.05) between the untreated and the GuardIVa® dressing test groups. After 7 days, the average wound treated with a Biopatch® dressing was found to be 34% healed. A statistically significant difference was observed (p<0.05) between the GuardIVa® dressing and the Biopatch® dressing test groups. The average percentage of wound healing at 7 days is found in Figure 7 below.

In Vivo Animal Study Wound Healing after 7 Days (Figure 7)

Conclusions
The in vivo dermal wound healing animal study performed by contract testing laboratories demonstrated that the GuardIVa® dressing healed at a comparable rate to untreated wounds. The Biopatch® dressing demonstrated a delay in healing when compared to untreated wounds and wounds treated with the GuardIVa® dressing.
Precautions

Bard® GuardIVa® Antimicrobial Hemostatic IV Dressing is not intended to treat infection.

Warnings

• Do not use the GuardIVa® dressing on patients with a known sensitivity to chlorhexidine gluconate. The use of chlorhexidine gluconate containing products has been reported to cause irritation, sensitization, and generalized allergic reactions. If any such reactions occur, discontinue use of the dressing immediately, and if severe, contact a physician.

• For external use only. Do not allow this product to contact ears, eyes, mouth or mucous membranes.

• Intended for single use. DO NOT REUSE. Reuse and/or repackaging may create a risk of patient or user infection, compromise the structural integrity and/or essential material and design characteristics of the device, which may lead to device failure, and/or lead to injury, illness or death of the patient.

References

1Kutzscher, L. Management of Irritant Contact Dermatitis and Peripherally Inserted Central Catheters. Clinical Journal of Oncology Nursing; April 2012; Vol 16, No. 2: pp. E48-E55


5Bhende, S., Rothenburger, S. In Vitro Antimicrobial Effectiveness of 5 Catheter Insertion-Site Dressings. Journal of the Association for Vascular Access; 2007; Vol. 12, No. 4; pp. 227-231
